Fed-batch production of the hydrophobins RodA and RodB from Aspergillus fumigatus in host Pichia pastoris

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***Objectives*:** *Aspergillus fumigatus* expresses the hydrophobins RodA and RodB on the surface of its conidia. RodA is known to be important for the pathogenesis of the fungus, but the role of RodB is unknown. The aim was to produce recombinant RodA and RodB for further characterication. ***Methods and materials*:** The genes encoding hydrophobins RodA and RodB was amplified by RT-PCR from the total RNA isolated from *A. fumigatus* (AF296 strain), and cloned into expression vectors pPICZαA and pPICZB while adding a C-terminal 6xHis-tag. The linearized plasmids were transformed into *P. pastoris* strain X33. The expression of the RodA and RodB genes was first studied in culture flasks in buffered complex methanol medium as protein production was dependent on the methanol-induced AOX1 promoter. Later production was scaled up to a 2 L fed-batch fermentor. Hydrophobins were purified using His-select Nickel Affinity gel. The emulsifying properties of recombinant hydrophobins were investigated using oil-water emulsions studied by light microscopy. ***Results*:** Protein bands of expected size were detected by SDS-PAGE and western blotting in the fermentation broth. Fed-batch production yielded approximately 300 mg/L. rRodB showed good emulsifying properties. ***Conclusion*:** RodA and RodB from *A. fumigatus* were successfully produced by yeast host *Pichia pastoris* with good yields.